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THE NASO-LABIAL GROOVE OF LUNGLESS SALAMANDERS.

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So far as I have been able to ascertain, there is no mention in scientific literature of a very definite external feature of certain salamanders to which, because of its location, I have given the name, *naso-labial groove*. This groove, which extends from the latero-ventral angle of the external naris in a practically vertical direction to the edge of the upper lip, I have found in the following species, a list which includes nearly every genus of the *Desmognathidæ* and *Plethodontidæ*, all, in fact, which I had the opportunity to examine:

PLETHODONTIDÆ.

Batrachoseps attenuatus, *Plethodon cinereus*,
Plethodon erythronotus, *Plethodon glutinosus*,
Gyrinophilus porphyriticus, *Manculus quadridigitatus*,
Spelerpes bilineatus, *Spelerpes longicaudus*,
Spelerpes guttolineatus, *Spelerpes ruber*,
Autodax lugubris,

DESMOGNATHIDÆ.

Desmognathus fusca, *Desmognathus brimlyorum*.

This feature is not only apparently characteristic of the *Plethodontidæ* and *Desmognathidæ*, but is evidently peculiar to these families. None of the representatives of the other families of the *Salamandrida*[1] which I have examined possess it, neither

[1] The species of salamanders in which I have found no naso-labial groove are as follows: *Salamandra maculosa*, *Triton cristatus*, *Triton alpestris*, *Triton helveticus*, *Diemyctylus viridescens*, *Amblystoma punctatum*, *A. opacum*, *A. talpoideum*, *A. jeffersonianum*, *Salamandrina perspicillata*.

have I been able to find in any of the *Derotremes* or the *Perennibranchs* any trace of it.

Moreover, since all of the species which are known to possess the groove are lungless, and, since of those which do not possess it only one, *Salamandrina perspicillata*, lacks functional lungs, it is evident that the groove has some function associated with respiratory habits and correlated with the absence of lungs.

Description of the Naso-Labial Groove and Associated Structures.

In my study of the naso-labial groove and the organs associated with it I have used mainly *Desmognathus fusca*, the species which I have been able to obtain in largest numbers, both in the adult and larval stages. The following description applies, therefore, particularly to this species.

The methods of study employed have included much observation of the living animals under varied conditions, gross dissection, and the microscopic study of both horizontal and transverse series of sections. Horizontal sections are particularly favorable for the study of the groove, since in these it is cut transversely.

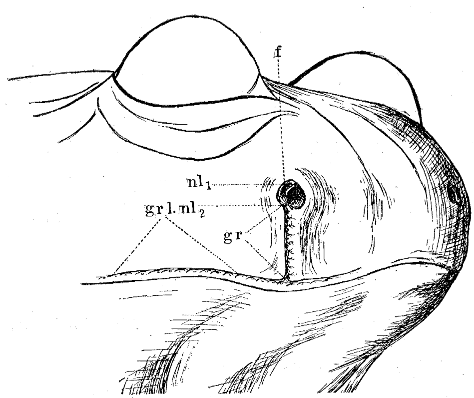

FIG. 1. Head of adult *Desmognathus fusca* showing external naris and naso-labial groove. *f*, crescentic fold; *gr*, naso-labial groove; *grl*, labial (horizontal) groove; nl_1 and nl_2, orifices of first and second naso-labial glands. Other orifices may be seen along the mesial side of the naso-labial groove and along the horizontal groove.

Externally the naso-labial groove (Fig. 1, *gr*), though a mere line, may be easily seen even with the unaided eye, its conspicu-

ousness being usually emphasized by the absence of pigment along its course. It begins at the latero-ventral angle of the external naris of which it is in a sense an exaggerated ventral elongation. From this point it extends ventrally until it reaches the edge of the lip, where it ends in a somewhat vaguely defined horizontal groove (Fig. 1, *grl*), which meets it at right angles. This horizontal groove is parallel with the edge of the lip and gradually disappears as it approaches the mid-line on the one hand and the angle of the mouth on the other.

In its connection with the external naris the naso-labial groove shows a very definite relationship to certain structures which have been worked out and described by Bruner ('96). This investigator has shown that the *Salamandrida* possess in connection with the external naris a set of involuntary muscles for opening and closing the orifice, the *M. constrictor naris* and *M. dilatator naris* respectively. The constrictor is situated in the lateral wall of the nasal cavity with its fibers partly encircling the orifice, so that externally the visible effect of its contraction is to draw a crescentic fold of skin (Fig. 1, *f*) across the orifice from the outer toward the inner border, the naris being thus partially or completely closed according to the amount of contraction. By the contraction of the dilatator muscle, the fold is again pulled back toward the outer boundary of the naris. When the orifice is closed the crescentic fold forms the floor of a slight depression which is completely encircled except at the latero-ventral angle, the point from which the naso-labial groove leads.

Associated with the external naris and with the naso-labial groove are a number of well developed glands which I shall speak of collectively as the *naso-labial glands*. Although these glands show much individual variation, the location of their orifices is practically constant for the species, the first two being specially definite. The more dorsal of these (Fig. 1, nl_1) is in the dorsal angle of the naris, that is, at the end of the crescentic fold. The second one (Fig. 1, nl_2) is on the ventral edge of the nasal orifice at the point where the groove joins the naris and therefore upon the mesial side of the groove. Arranged at approximately regular intervals along the mesial border of the groove are other orifices, from six to twelve in number, and similar orifices occur

along the ventral border of the horizontal groove into which the naso-labial groove leads. The orifices associated with the grooves are usually visible externally through a lens, since each is situated in the middle of a little elevation.

Seen in transverse section, that is, in a series of horizontal sections through the head, the naso-labial groove appears as an infolding of the entire epidermis (Figs. 2, 3 and 4, *gr*), the deeper

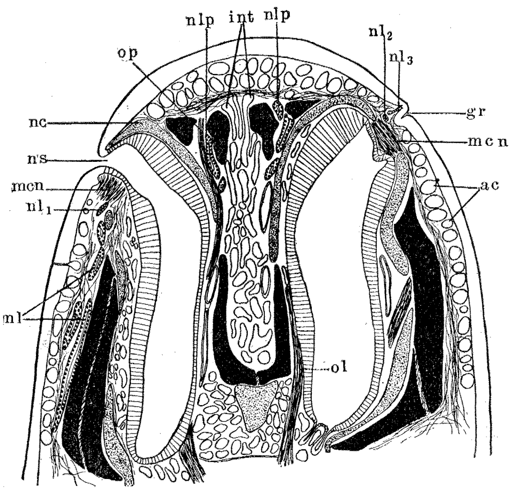

FIG. 2. Horizontal section through head of adult *Desmognathus fusca*, × 20. *ac*, acinous glands of skin ; *gr*, cross-section of naso-labial groove ; *int*, intermaxillary glands ; *mcn*, M. constrictor naris ; *nc*, nasal capsule ; *nl*, naso-labial glands ; nl_1, nl_2, nl_3, the first, second and third naso-labial glands respectively ; *nlp*, tubules of naso-labial glands within the premaxillary foramina ; *ns*, external naris ; *ol*, olfactory nerve ; *op*, internal nasal branch of ophthalmic nerve.

portion of which has a curved outline, while the outer portion dips abruptly in, giving the groove in transverse section the appearance of the letter V. It is deeper and narrower near the naris and becomes wider toward the edge of the lip. Underlying the groove is a dense mass of connective tissue (Fig. 3, *c*) richly supplied with blood vessels.

In these horizontal sections the naso-labial glands (*nl*) are seen to make a vertical descent through the epidermis and then to curve sharply and extend in either a general mesial or lateral direction beneath the skin and parallel with it. Although it is

often difficult to distinguish in sections between the narrow ducts of these glands and those of the large acinous glands (*ac*) with

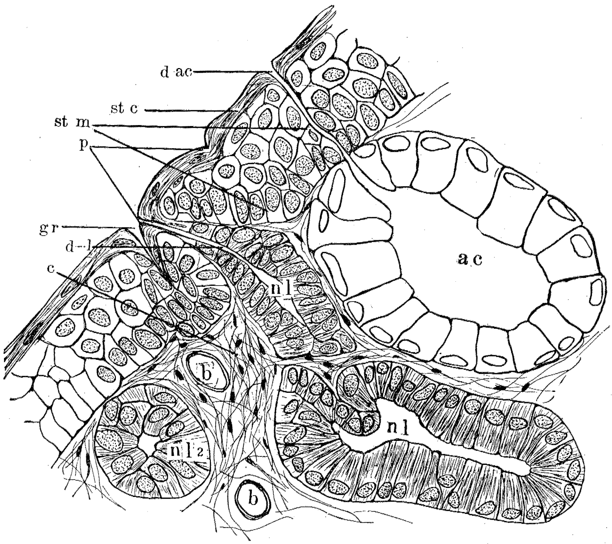

FIG. 3. Transverse section through left naso-labial groove of adult *Desmognathus fusca*, × 237.5. *b*, blood vessels; *c*, connective tissue underlying the naso-labial groove; *dac*, duct of acinous gland; *dnl*, duct of naso-labial gland; *p*, papilla upon the surface of which the naso-labial gland opens; *stc*, stratum corneum; *stm*, stratum mucosum. Other designations as in Fig. 2.

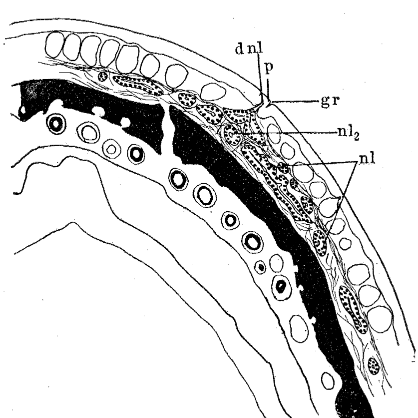

FIG. 4. Horizontal section through right side of head of a small adult, *Desmognathus fusca*, × 42. Designations as in previous figures. The second gland (nl_2) is cut through the portion which runs for a short distance parallel to the groove and just beneath it (cf. Fig. 5).

which the skin is thickly studded, the glandular portions are readily distinguished by the smaller size of the naso-labial glands and of their lumina, as well as by their deeper situation. The acinous glands are a part of the skin and lie within it; the naso-labial lie beneath the skin.

In working out the course of the glands two methods were used. The more reliable one as to exact relationships was the serial-section method by means of which the complete course of each tubule may be worked out. Several series both horizontal and transverse were used in this way. The disadvantage of this method lies in the fact that since the glands are of small diameter, much convoluted, and in some regions closely packed together, it is necessary, in order to obtain reliable results, to have thin sections and an absolutely uninterrupted series, conditions which are not easily fulfilled in sectioning an entire head. Even with a

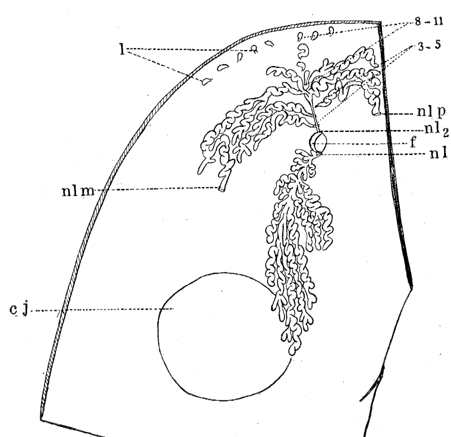

FIG. 5. Dissection of individual shown in Fig. 1, in which the skin is removed from the right side of the head, the naso-labial glands being removed with it. Drawing shows under surface of skin with glands in place. *cj*, conjunctiva; *l*, labial glands; 3–5 and 8–11 indicate the enumeration of the naso-labial glands; *nlm*, cut end of tubule which lies in the groove of the maxillary bone. Other designations as in previous figures.

perfect series, moreover, it is extremely laborious to trace even a single tubule throughout its tortuous course. Gross dissections were, therefore, found to be of great value as a means of corrob-

oration of results obtained from the study of serial sections. It was found possible, with alcoholic specimens, to dissect off the skin from the entire head, removing with it all of the soft parts, eyeballs included, external to the skull; and such dissections, while always involving more or less injury to some of the tubules, were still sufficiently good so that, when the skin was pinned out under water and further dissection and separation of the glands accomplished, practically every anatomical fact which had been learned from the sections could be demonstrated.

The naso-labial glands are tubular glands much convoluted, and showing a tendency to develop acini at the bends of the tubules. The different glands of the group show great inequality both in complexity and in length of the tubules. The longest and most complicated are the two which are associated with the naris itself [referred to as (nl_1) and (nl_2) in the figures]. Both of these are not only much convoluted but are branching. The dorsal gland (nl_1) often sends several branches as far posteriorly as the orbit, over the edge of which a few tubules usually find their way, while some may even extend entirely across the inner surface of the eyeball. The duct of the second gland (nl_2) extends ventrally for a little distance beneath the groove, and then gives off almost at right angles several branches both mesial and lateral. The lateral have been in some specimens traced in a course parallel with the edge of the lip as far posteriorly as the eyeball, while the mesial extend nearly to the mid-line and then may turn in a dorsal direction.

Of the remaining naso-labial glands (Fig. 5, *3–5*, *8–11*), some are several millimeters in length, others much shorter. All, however, are long enough to be classified as tubular glands. The shorter glands are in general those opening along the more ventral portion of the groove, and along the horizontal groove (*grl*) only very short ones have been observed. All of the longer tubules lie beneath the skin parallel with the edge of the lip, extending either mesially or laterally from the groove, and they sometimes equal in length the single branches of the large gland (nl_2) which opens on the ventral border of the naris. Occasionally a mesial and a lateral tubule unite and open by a single duct (Fig. 4, *dnl*), thus forming a branching gland.

In two regions the naso-labial glands come into close proximity with other glands of such similar structure that the determination of the actual relationship is very perplexing. One, at least, of the mesial tubules (Figs. 2 and 6, *nlp*),[1] turns dorsally

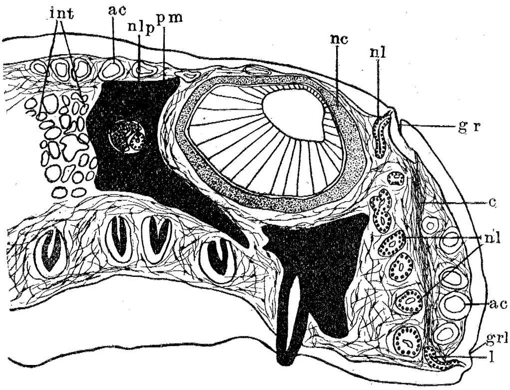

FIG. 6. Transverse section through head of adult *Desmognathus fusca* (× 42), showing the naso-labial groove cut obliquely and the horizontal groove (*grl*) cut transversely. *pm*, premaxillary bone. Other designations as in previous figures.

before reaching the mid-line and enters the foramen for the internal nasal branch of the ophthalmic nerve at the anterior end of the premaxillary bone. Through this foramen the tubule may be traced as far as the opening of the foramen into the intermaxillary space. In this space lie the much convoluted tubules of the intermaxillary gland (*int*), the orifice of which is in the mid-line of the roof of the mouth, a little anterior to the posterior nares. In the larger specimens the naso-labial tubule passes into the intermaxillary space and intermingles its convolutions with those of the intermaxillary gland. I have found it impossible from my preparations to determine whether the tubules of the two glands thus curiously intertwined become actually continuous or not. If such an anastomosis exists, however, it is certainly established secondarily, for in small adults the naso-labial tubule has not reached the intermaxillary space but ends blindly within the foramen, and there is consequently

[1] In every case in which I have been able to demonstrate the relationships of this tubule with absolute certainty, it has proved to be one of the branches of the gland which opens on the ventral margin of the naris (nl_2).

here an unmistakable interval between the two glandular masses.

The other region in which one of the naso-labial glands may become closely related in position to other glands is in the lower part of the orbit where there is a large glandular mass which opens by several orifices on the inner surface of the lower lid. These glands I shall speak of collectively as the *orbital glands*. The tubules of these glands, following the same principles of growth along lines of least resistance as do the naso-labial glands, push their way around both the anterior and the posterior angles of the orbit. The anterior orbital glands and the ultimate branches of the most dorsal naso-labial gland may, therefore, lie side by side within the orbit behind the eyeball. In no case, however, has any trace of coalescence been observed; the tubules of each gland end blindly. Here, again, the condition in the small adults substantiates the conclusion that the association of the glands is merely one of proximity, since in the young adults the naso-labial gland has not reached the orbit and therefore its tubules do not even lie beside those of the orbital gland.

This peculiar relation of the naso-labial to other glands has, therefore, in all probability no significance whatever beyond the fact that it is the tendency of all these glands to work their way into whatever crevice happens to lie in their course. Thus certain of the lateral tubules of the naso-labial glands (Fig. 5, *nlm*) usually find out the foramen in the maxillary bone for the external nasal branch of the ophthalmic nerve and enter it; and the same tendency is shown also by the intermaxillary gland, some of the tubules of which escape from the intermaxillary space through the anterior fontanelle and extend anteriorly immediately beneath the skin of the snout, while other tubules of the same gland escape through the nerve foramen which leads back into the posterior part of the nasal capsule, and thus creep into the cavity under the nasal epithelium in which the internal nasal glands are located.

So far as can be seen externally the naso-labial groove in other species does not differ essentially from that of *Desmognathus fusca*. The relation to the external naris is always the same, but the method of termination at the edge of the lip varies

somewhat. Thus in *Plethedon* the groove gradually becomes less distinct and finally flattens out and is lost upon the surface of a slight labial eminence.

I have worked out the glands by dissection in several species. They all show the same general character, and the position of the orifices of two especially large glands with relation to the naris is practically constant and similar to that in *Desmognathus* (nl_1 and nl_2 of the figures).

The location of the remainder of the orifices is difficult to determine. The position along the mesial border of the naso-labial groove, while a common one, does not seem to hold exclusive possession in all species. The glands do, however, open always in close proximity to the groove, and so far as I have been able to determine, never within it. From the groove the glands have the same mesial and lateral course, parallel with the lip. Some of the glands are always extensive, usually reaching nearly or quite as far posteriorly as the eye. In *Plethodon* several of the

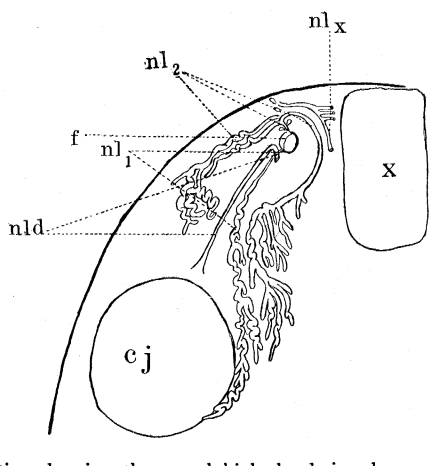

FIG. 7. Dissection showing the naso-labial glands in place on under surface of skin removed from the right side of the head of an adult *Plethodon*. *X*, the area occupied by a mass of tubules partly from the intermaxillary gland and partly from the naso-labial (nl_x). Other designations as in previous figures.

mesial tubules (Fig. 7, *nlx*) turn dorsally before reaching the midline and extend posteriorly over the surface of the snout, intermingle with the intermaxillary, the tubules of which extend

out through the anterior fontanelle, and finally send several tubules laterally as far back as the dorsal edge of the orbit.

The naso-labial glands are in no sense a new discovery. Under various names they have been described at least in part by Wiedersheim ('76), Seydel ('95), Born ('77) and Bruner ('96 and '01). Wiedersheim ('76) in particular mentions the enormous extent of large external glandular masses in various species of *Plethodon*, *Spelerpes*, *Batrachoseps*, *Gymnophilus* (*Gyrinophilus ?*) and *Chioglossa*. It is interesting to note with regard to this list, made eighteen years before the discovery that any of the salamanders are lungless, that the first four of the genera are now known to be lungless while so far as I know the fifth, *Chioglossa*, has not been investigated as to a possible lungless condition.

Other investigators, particularly Bruner ('96) mention under the discussion of *external nasal glands* in general, some of the *Plethodontidæ* and *Desmognathidæ* as genera in which such glands occur. The glands to which Bruner referred were undoubtedly the two of the naso-labial group which open in connection with the naris. I cannot find in these authors, however, any indication of an appreciation of the great difference in size and importance between these glands of lungless forms and those similarly named which occur in the lunged forms. Bruner, in fact, in discussing the function of the external nasal glands mentions particularly that the extent of the glands exceeds very little that of the *apertura nasalis externis*, a fact which is in general true of the lunged salamanders but is, as has been shown above, far from true in the case of the members of the lungless families. Moreover, the course of the glands in the lunged species which I have examined lies between the nasal capsule and the maxillary bones, while in the *Desmognathidæ* and *Plethodontidæ* these glands are in the main external to all the skeletal parts.

With regard also to the exact location of the orifices of these naso-labial glands the facts seem not to have been clearly and completely stated. Wiedersheim ('76), indeed, located the orifices of the glands in *Batrachoseps* and *Spelerpes* upon the border of the upper lip. He was probably misled, however, by the close association of the ultimate gland tubules with those of the

orbital glands, into the opinion that in *Batrachoseps* at least, the glandular mass had orifices upon the surface of the conjunctiva also. I have not myself sectioned *Batrachoseps* but from the relationships which I have worked out from sections of both *Desmognathus* and *Plethodon* I am convinced that the orbital glands are absolutely distinct and separate from the naso-labial in these forms.

Bruner's ('96, *b*) observations as to the orifices of the glands were correct so far as they went in that these were located in common with those of the external nasal glands of other salamanders, on the border of the naris. His observations, however, did not extend to those of the glands which open along the naso-labial groove.

Development of the Naso-Labial Groove and Glands.

The naso-labial apparatus is essentially an adult characteristic. In large *Desmognathus* larvæ (25 mm.) collected in early summer and only a few millimeters shorter than the smallest adults which I have found (33 mm.) the groove has hardly begun to develop and the anlagen of only the largest of the glands are to be found. During the two months which follow, these structures undergo rapid development, so that the little adults which abound

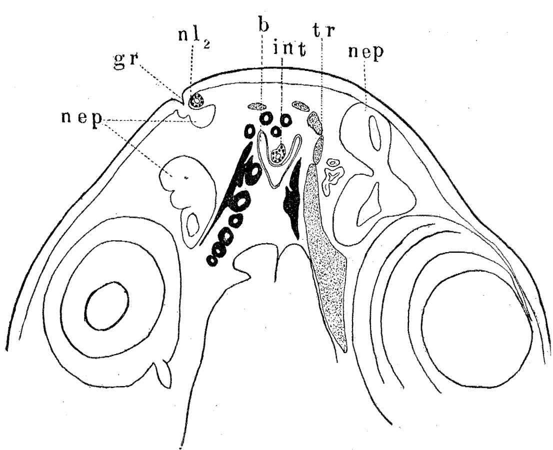

Fig. 8. Horizontal section through the head of a *Desmognathus fusca* larva, cut slightly obliquely. The left side of the section passes just below, the right slightly above, the external naris, × 42. *nep*, nasal epithelium; *tr*, trabecula. Other designations as in previous figures.

in early autumn along the banks of the brooks in which the larvæ live, have developed, with the assumption of terrestrial habits, these distinctively adult characteristics, namely, naso-labial grooves and well developed glands opening in connection with them.

There exist, however, even in the larvæ, certain indications that the process of formation of a naso-labial groove has begun. There is a ventral elongation of the external naris, which really forms a very short groove (Fig. 8, *gr.*). Ventral to this there may be seen by following the horizontal sections for a short distance a slight incurving of the epidermis with a tendency here and there toward the proliferation of cells (*pr*) of the lower of the two layers, a suggestion, at least, of the beginnings of future

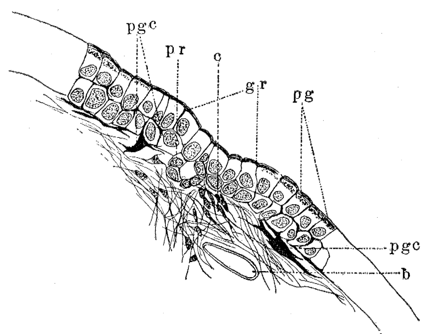

FIG. 9. Detail of a horizontal section through the head of a *Desmognathus fusca* larva ventral to the external naris, showing an early stage in the formation of the naso-labial groove, $\times$ 237.5. *pg*, pigment within the cells of the epidermis; *pgc*, pigment cells below and between the cells of the epidermis. Both forms of pigment are absent from the region in which the groove is developing (*gr*) *pr*, proliferation of cells from the under side of the epidermis, probably the anlage of a gland. Other designations as in previous figures.

glands. Noticeable is the absence of pigment in this region both in the case of the epidermal cells and the connective tissue beneath the epidermis. The first glands to appear, and at this stage the *only* ones present, are the two connected with the naris. These are very short, the dorsal extends posteriorly through three or four 10μ sections, the ventral one extends mesially and is very probably the branch which later finds its way into the intermaxillary space.

The small adults, 33 to 45 mm., have the groove well developed. The number of glands connected with it seems to increase

gradually as the animals grow older. The smallest showed three or four only, the more ventral of these being very short. A specimen 43.5 mm. long had six in connection with each groove, while in a large adult as many as twelve are often present.

The extent of the glands also increases as the animals grow larger, a fact which has already been mentioned as throwing light upon the relation of the glands to other glandular masses. One very interesting fact is that the tubule which enters the nerve foramen in the premaxillary does this previous to the roofing over of the foramen. It thus lies at first on the surface of the premaxillary in a mere groove which later becomes enclosed to form a foramen.

The Homology of the Naso-Labial Glands.

There seems to be no reason why the homology which Bruner ('96) has assumed to exist between the external nasal glands of all of the *Salamandrida* and *Anura* should not be accepted for the *Desmognathidæ* and *Plethodontidæ*, in spite of the greater extent and more external position of the glands in these families. This homology will of course include strictly only those two of naso-labial group which open upon the border of the naris, and is based upon similarity of origin and structure between these and the external nasal glands of lunged forms. There is, however, absolutely no reason for considering the remainder of the naso-labial glands as in any essential unlike these two, and the entire group must, of course, be homologized together.

Moreover, I have obtained sufficient evidence (which will be given in a separate paper) to prove that the large gland which occurs in *Amphiuma* and was described by H. H. Wilder ('91) under the name of the "*glandula lateralis*," is also the homologue of these glands as well as of *Oppel's* glands of *Proteus*. The homology of all these glands becomes especially emphasized when we recall that in many of the lunged forms the external nasal glands consist of many tubules opening by separate orifices. Thus Bruner reports for one specimen of *Amblystoma* fifteen independent tubules.

To go back of the adult condition and determine the source from which these external nasal and naso-labial glands were

originally derived, and to decide the question as to their possible relation in origin to other glandular structures such as the acinous glands must, however, be left to further investigation.

The Function of the Naso-Labial Groove and Its Associated Glands.

As expressed in the introductory paragraph, the exclusive occurrence of so definite and well developed a set of organs as the naso-labial groove and glands in those families which are characterized by absence of lungs must be correlated with the lungless condition. The key to the explanation of their functions will be found, therefore, in a study of the respiratory habits, particularly those which involve a use of the nasal passages.

Although certain species of the *Plethodontidæ* and *Desmognathidæ* have an aquatic larval life, practically all are terrestrial in the adult stage. They live in moist places under stones and decaying wood or, like *Desmognathus*, burrow in loose, moist earth along the banks of small streams. Some species, among them *Desmognathus fusca*, do not even go into the water to deposit their eggs. *Autodax lugubris* has carried its terrestrial habits to such an extreme that it has become arboreal, and rears its young in the cavities of tree trunks [Ritter and Miller ('99) and Ritter ('03)]. I have been able to find in the stomachs of both *Desmognathus fusca* and *Plethodon cinereus* only spiders, mites and terrestrial insects, and I am led to infer that the food of these species consists wholly of terrestrial forms.

Whatever other methods of respiration the lungless salamanders may have, they maintain, so long as they have access to air, a constant bucco-pharyngeal aërial respiration. This is approximately regular and is very rapid, sometimes exceeding a hundred respirations a minute, although the number may be as low as fifty. It is accomplished by an alternate depression and elevation of the floor of the tightly closed mouth, as a result of which air is drawn in and expelled through the nasal passages. Slight occasional fluctuations of the crescentic fold which guards the external naris may be observed, but, under ordinary conditions, not a complete closure. If, however, the specimen be immersed in water, or if a drop of water be applied to the snout so

as to flow over the external nares, these immediately close, while bucco-pharyngeal respiration at once ceases and the floor of the mouth is forcibly indrawn, causing, often, the expulsion of tiny bubbles of air through the nares which open sufficiently to permit their escape. All entrance of water into the nasal passages is, by this prompt closing of the nares, effectually prevented. There is, moreover, an independent control of the two nares, since, when the animal is so manipulated that only one naris is covered with water, this one alone is closed, while, through the other, aërial respiration continues. Especially noticeable is the almost immediate resumption of aërial respiration, when, after the interruption caused by immersion, the snout is thrust out of water sufficiently to bring the nares above its surface. Even if only a single naris gains access to the air, this alone opens and the bucco-pharyngeal movements begin again.

All of the forms which I have experimented with, however (*i. e.*, various species of *Plethodon*, *Desmognathus* and *Spelerpes*), show, with the exception of *Spelerpes ruber*, a strong aversion to deep water. When placed in water of too great depth to allow them to raise the tip of the snout above the surface while they are standing upon the bottom, they invariably make frantic efforts to escape. They swim rapidly to the top and hold themselves there by active swimming motions. When unable to sustain themselves longer at the surface, since, in the absence of lungs, they have no hydrostatic powers, they fall to the bottom where they remain for a longer or shorter period of rest. During all these manœuvres the tightly closed condition of the nares and the elevated position of the floor of the mouth are maintained. Moreover, from my observation of *Desmognathus fusca* and *Spelerpes bilineatus* in their natural environment, I am convinced that these species, at least, never voluntarily seek deep water; and when driven into deeper parts of the stream, they soon make their way under cover of fallen leaves and débris to a region where the depth is not too great to allow the snout to be thrust out of water. Under this condition they are perfectly at ease and will remain with the body in water for a long time if only the nares have access to the air so that bucco-pharyngeal respiration may continue.

It is, however, an interesting fact that although bucco-pharyngeal aërial respiration may be necessary to the comfort of these lungless forms, it is apparently not an absolute physiological necessity. I made the experiment of confining individuals representing several species of *Desmognathus*, *Plethodon* and *Spelerpes* in wire cages which were kept immersed in running water with no possible access to air for periods ranging from one to two weeks in length. Although when liberated at the end of the experiment they invariably swam immediately to the surface and made the same vigorous efforts to escape that they had made previous to the experiment, they showed no signs of impairment of physical function and continued to live afterwards in the terrarium with no evidence of ill effects from the ordeal. They were never discovered, however, to open the nares during the confinement under water, or to show any other than the tightly indrawn position of the floor of the mouth.

Spelerpes ruber, unlike most other lungless forms, shows a a decided adaptability to aquatic life, and will live for weeks at the bottom of an aquarium. In this case, however, as in those of less aquatic forms, under compulsory immersion, it is a significant fact that *not only were the external nares apparently kept tightly closed so long as the animal was under the water*, but when finally removed from the water and given access to the air *the resumption of aërial bucco-pharyngeal respiration was immediate.*

The importance of these points for the present discussion will be emphasized by comparison with the habits and behavior of lunged salamanders under similar conditions. All of the lunged salamanders have, in addition to pulmonary respiration, a bucco-pharyngeal aërial respiration exactly similar to that of the lungless forms. When these lunged salamanders are placed under water, however, although there is at first an attempt to exclude the water from the nasal passages by closure of the external nares, these are sooner or later opened and aquatic bucco-pharyngeal respiration is established similar mechanically to the aërial method, though much slower. Usually there is the additional difference that the water is expelled in part through the slightly opened mouth, and in some few instances it has been observed to enter, also, through this channel as well as through the nares.

The efficiency and readiness of this aquatic bucco-pharyngeal respiration varies much with the aquatic habits of the individual species, *Diemyctylus viridescens*, which is thoroughly aquatic, showing one extreme, *Amblystoma opacum*[1] the other.

After the establishment of aquatic bucco-pharyngeal respiration in these forms, the removal from water to air is not accompanied, as in the case of the lungless salamanders, by an immediate resumption of aërial respiration. On the contrary there is often much delay arising from the mechanical inconvenience of having filled the nasal passages with water. It frequently requires much gasping and several enormously exaggerated depressions of the floor of the mouth as if an attempt were being made to draw an obstruction from the nasal passages before finally unimpeded aërial respiration through these is reëstablished.

With this difference between the respiratory habits of lunged and lungless forms clearly in mind we are ready for the explanation of the part which the naso-labial groove and glands play in making possible the immediate resumption of aërial respiration after its temporary interruption by submersion in water.

When the nares are closed, there remains, externally, a slight depression, the floor of which is formed by the fold which closes the orifice. When the snout is thrust out of water there is a tendency, as the water drains from the surface of the head, for each nasal depression to retain some of it. Thus it would be expected that the opening of the naris for the resumption of aërial respiration would allow this water to enter the nasal passage, especially as the depression of the floor of the mouth in the act of inhaling would draw in the water first of all. As a matter of fact, however, nothing of the sort occurs. When the snout is thrust out of water, the accumulation of the fluid in the nasal depression is only momentary. With the opening of the naris through the drawing back of the crescentic fold, the last vestige of this drop of water disappears ; and, although the whole surrounding skin is wet, the nasal depression and the naso-labial

[1] In a previous article on the Ypsiloid Apparatus of Urodeles (BIOLOGICAL BULLETIN, Vol. X., No. 6), I have shown that this species, which has been erroneously supposed to be lungless, really has well developed, functional lungs. In the same article is given also a more complete account of the respiratory habits of the lunged salamanders.

groove may be seen, by the aid of a lens, to be absolutely free from water which, moreover, *recedes slightly from the border of both naris and groove.* Although the general mechanical process by which the naris is thus quickly freed from water is obviously through the medium of the groove, the absolute determination, experimentally, of the physical principles involved in the performance of the function of the groove and glands proved somewhat difficult. The chief obstacles lie in the fact that the parts concerned are so extremely small that the experiments must be made under a lens of considerable magnifying power; while the animals themselves, which, of course, must be in their normal, active condition, are extremely agile and object strenuously to the experimenting process. The most effective device was found to be that of holding the specimen wrapped in a slightly moistened handkerchief with only the head protruding. Specimens handled in this way, however, soon become too much dried to react normally and must frequently be returned to the terrarium and a fresh specimen taken. Although many experiments were made, with varying degrees of success, the following set of experiments presents all of the facts from which the final conclusions were drawn. These experiments or slight variations of them were repeated many times and with different individuals, always with virtually the same results.

Experiment 1. — The head of the specimen was thoroughly dried by means of absorbent paper. The nares were open and normal aërial respiration was taking place. A small glass tube, one end of which was drawn out to a fine capillary point, was partly filled with water. By blowing into the larger end of the tube a tiny drop could be forced out through the capillary end. This drop was deposited on one of the nares, the object being to get a drop small enough to exactly fill the nasal depression. The result was the immediate closure of the naris and a subsequent immediate reopening, since the water disappeared at once. The experiment was repeated with a drop of diluted India ink substituted for the water. At once the groove became filled with the ink, which accumulated in a little drop at the labial end where it flowed off over the surface of the skin. The naris again reopened immediately, completely free from the fluid. The mouth was

then forced open and the region about the posterior nares examined to see if by any chance the ink had entered the naris. No trace of it was found, however, anywhere in the mouth cavity. This portion of the experiment shows, what of course the experiment with water had failed to show conclusively, that *the groove is the sole channel through which the fluid escapes from the naris.* The groove, moreover, retained a tiny line of the ink after the naris was free from it, and the ink remained there until a subsequent application of water to the naris resulted in the removal of the ink as the water took its place in the groove.

Experiment 2. — The head of the specimen used in this experiment was wet by allowing water to flow over it from a pipette. When the nares were opened and normal aërial respiration was established, the attempt was made to place a small drop of ink in the nasal depression. This proved difficult because the film of water which covered the surrounding area attracted the drop and the ink would flow off over the surface of the skin instead of entering the nasal depression. When finally a drop was successfully placed upon the naris, there was an immediate appearance of a line of ink filling the groove and escaping from its lower end. After a moment the naris opened as before, wholly free from the fluid. This experiment not only corroborates the conclusions drawn from the first experiment as to the function of the groove, but shows that *under the usual moist condition of the skin there is a tendency for a small drop of water falling upon the snout not to flood the nasal depression at all but to mingle with the general moisture covering the skin and to flow off over the surface.* This tendency is referable to that condition of the edge of the naris which causes the water to recede slightly from the orifice while it freely wets the neighboring skin and remains as a thin film upon its surface.

Experiment 3. — The head of the specimen was dried and a drop of ink was applied to the edge of the lip at the labial end of the groove. The groove at once filled with ink which extended *up* toward the naris. Upon reaching it, however, it produced no interference with respiration for it merely filled the wrinkles in the external surface of the crescentic fold which was drawn back to the outer edge of the orifice. *The experiment*

thus demonstrated with absolute certainly the capillary action of the groove and at the same time showed that *the relation of the groove to the fold renders it impossible for the naris to become flooded through this capillary action of the groove* even when the lower end of the latter is in the water, a common occurrence under normal conditions.

Experiment 4. — The head of the specimen was thoroughly dried with absorbent paper and, when normal respiration through the open nares was in progress, a thin film of olive oil in which lamp black was suspended was quickly spread over the whole surface of the head. There was the customary opening of the naris by the withdrawal of the crescentic fold, but *this was unaccompanied by the usual receding of the fluid from the edge of the naris and the draining off of the naris through the groove.* The groove seemed wholly obliterated by the oil, which formed a continuous film over it. A line of lamp black could, however, be seen slowly settling to the bottom of the groove, showing that the oil had entered it. The oil extended to the very edge of the nasal orifice and with each often repeated closure of the naris the nasal depression could be seen to fill again with the oil. The animal made frequent efforts to rub off the oil and finally being unsuccessful in freeing the nares of the oil it slightly opened its mouth and attempted to resume bucco-pharyngeal respiration through this new channel. Upon examining the interior of the mouth I could see that the black oil had passed through the posterior nares and had entered the mouth.

This experiment showed that the condition of the skin bordering the naris and groove, although such as to slightly repel water, did not have this effect upon the oil which could not, therefore, be drained from the naris but overflowed its edges and entered the nasal passages. This indicates that *the secretion of the naso-labial glands mixes with oil but repels water and that it is essential to the successful performance of the function of the groove.*

Experiment 5. — In this experiment a drop of water in which carmine was suspended was placed upon the naris. The particles of carmine were evidently too large for the groove, since there was no filling of the groove by the fluid. After a moment, however, there was a sudden disappearance of the larger part of

the drop. Evidently the crescentic fold had been withdrawn as usual but the cessation of the usual function of the groove had resulted in the fluid entering the naris. This inference was proved correct by an examination of the interior of the mouth in which the carmine was found.

In the light of these experiments the process of freeing the nasal depression from water may be explained as follows:

When, after the excess of water has flowed off from the general surface of the head, a small amount is left in the nasal depression, its surface tension is too great to allow its removal merely in obedience to gravitation. There is, therefore, a most delicately adjusted combination of devices by means of which gravitation is aided and the rapid removal of the water from the naris is effected. These are (1) the capillarity of the groove which is, especially at its nasal end, very narrow and deep; (2) the continuity through the medium of the groove, of the water in the nasal depression with the water at the labial end of the groove, even though this may be merely a larger drop which has accumulated on the surface of the lip; (3) the final impetus to flow down the groove, given by the wrinkling back of the crescentic fold in the process of opening the naris. It is impossible to say which of these factors is of chief importance. When the amount of water is very small, probably either the first or the last alone would suffice. With a larger amount of water the deeper portion of the groove quickly fills by capillary attraction and then its outer, wider portion probably serves merely as a gutter down which the water flows by gravitation. That the capillarity of the groove, working in the reverse direction, does not prove a disadvantage is due to the fact that the groove is continuous with the external surface of the crescentic fold which is, therefore, the only part affected.

Although the nature of the secretion of the glands has not been chemically tested, their function is obvious. The only portion of the head over which water does not flow freely and from which water seems in fact to be repelled is the region immediately surrounding the naris and the mesial border of the groove. In these regions microscopic sections show a noticeable lack of the large acinous glands of the skin, the secretion of which readily mixes

with water. These regions, on the other hand, are in the immediate vicinity of the orifices of the naso-labial glands. The motions of the crescentic fold are observable not only in opening and closing the naris, but to a lesser degree as frequent slight fluctuations. Undoubtedly these not only stimulate the flow of the secretions of the two large glands associated with the naris [cf. Bruner ('01)], but also effect the spreading of the secretion around the margin of the orifice. Furthermore, the orifices of the glands which are associated with the groove are elevated above the groove by their location upon papillæ. Thus while their secretion is discharged along the edge of the groove, the groove itself is kept wholly free from it. This is a necessary provision since because of the power of the secretion to repel water, it would, if it entered the groove itself, destroy its capillary power to remove water from the nasal depression. The nature of the secretion is probably such that it spreads easily upon the surface of the skin and thus effects the repulsion of water from the margin of the naris and groove. This function of the secretion is very important in two ways, first, it serves as a means of preventing the frequent flooding of the nasal depression from contact with drops of dew, for example; and, second, after the flooding of the naris has occurred, it acts as a means for quickly shutting off the water which lodges in the nasal depression and groove from the film of water upon the surrounding surface of the skin and thus makes it possible for the groove to remove the residue of water from the nasal depression. The large size of these glands proves that their function is one of great importance.

As to the purpose of so delicately adjusted and efficient a mechanism for quickly freeing the nares from water, one can only conjecture. It is undoubtedly connected with the apparently universal habit among the *Plethodontidæ* and *Desmognathidæ* of maintaining a dry condition of the nasal passages. That the ability to reëstablish aërial respiration so promptly is primarily because of the importance for that particular mode of respiration seems, however, to be disproved by the experiment above cited in which specimens lived for many days immersed in water with no access to air. Neither was it found in any way injurious to artificially fill both the nasal passage and the whole mouth cavity with

water, an experiment which I made by blowing water in through a capillary tube inserted into the naris. The individual thus treated merely experienced considerable inconvenience for a few minutes in his efforts to remove the water from the nasal passages, an end which he finally accomplished by swallowing forcibly. After a few minutes he was breathing air as usual.

I am convinced, however, that the respiratory functions of lungless salamanders are not as yet fully understood and it is very probable that further experimentation may reveal conditions under which the ability to promptly resume aërial respiration is a matter of great importance.

Another possibility suggests itself as an explanation of the need for the naso-labial apparatus. These animals are nocturnal in their activities and it is probable that in their search for food they are largely dependent upon the sense of smell. If so, their successful search would be much interferred with by even a temporary cessation of bucco-pharyngeal respiration, since it is by means of this act that the odors reach the nasal epithelium. In view of this supposition a device for quickly freeing the nares of water would be of the greatest importance, since in these nocturnal excursions the animal must be constantly wetting the head with drops of dew.

The question naturally arises as to the conditions in the one species, *Salamandrina perspicillata*, which is known to be lungless and yet to possess no naso-labial groove. I have unfortunately been unable to obtain living specimens of this European form. It is said, however, to have a bucco-pharyngeal *aquatic* respiration and in that case would not be expected to have a highly specialized apparatus for maintaining a dry condition of the nasal passages. In all events, its lungless state was acquired more recently than that of the *Plethodontidæ* and *Desmognathidæ* and quite independently of them, and may have been the result of very different conditions.

Throughout the investigations involved in this article I have been much helped by the advice and criticism of Dr. H. H. Wilder, and I wish here to express my gratitude to him for the assistance thus rendered to me.

CONCLUSIONS.

1. The naso-labial groove is a characteristic of the adult stage of the *Plethodontidæ* and *Desmognathidæ*.

2. Associated with the naso-labial groove is a system of glands which lie beneath the skin, and, in the main, external to all skeletal structures and open upon the border of the naris and groove.

3. These glands are homologous with the external nasal glands of other *Salamandrida* and of the *Anura*, but in the lungless families certain of them attain an enormous development.

4. The function of the naso-labial apparatus is to quickly remove from the nasal depression the small quantity of water which collects there when the head has been flooded with water. The object of this prompt removal of water is to permit an immediate resumption of aërial bucco-pharyngeal respiration after its temporary interruption by immersion in water.

5. The function of the glands is to guard both the naris and the groove against a continual overflow of water from the neighboring wet surface of the skin. The secretion is of a nature to repel water and by its agency water lodged in the nasal depression is cut off from that on the surrounding surface and can therefore be removed through the groove.

6. The groove performs its function partly through its capillary action, thus overcoming the surface tension when the quantity of water to be removed is very small; and partly merely as a gutter permitting the flow of the fluid from the naris to the lip. In its function it is assisted by the motion of the crescentic fold of skin which is drawn across the naris to close it against the entrance of water. The withdrawal of this fold tends to push the water out of the depression into the groove.

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BIBLIOGRAPHY.

Born, G.

'77 Über die Nasenhöhlen und den Thränennasengang der Amphibien. Morph. Jahrb., Bd. II.

Bruner, H. I.

'96 Ein neuer Muskelapparat zum Schliessen und Öffnen der Nasenlöcher bei den Salamandriden. Archiv für Anat. und Phys. Anat. Abth.

Bruner, H. I.
'01 The Smooth Facial Muscles of Anura and Salamandrina. Morph. Jahrb., Bd. XXIX.

Oppel, A.
'89 Beiträge zur Anatomie des Proteus anguineus. Archiv. f. Mikros. Anat., Bd. XXXIV.

Riese, H.
'91 Beiträge zur Anatomie des Tylotriton verrucosus. Zool. Jahrb., Bd. V.

Ritter, Wm. E., and Miller, L. H.
'99 A Contribution to the Life History of Autodax lugubris (Hallow), a California Salamander. Amer. Nat., Vol. XXXIII., No. 393.

Ritter, Wm. E.
'03 Further Notes on the Habits of Autodax lugubris. Amer. Nat., Vol. XXXVII., No. 444.

Seydel, O.
'95 Über die Nasenhöhle und das Jacobson'che Organ der Amphibien. Morph. Jahrb., Bd. XXIII.

Wiedersheim, R.
'76 Die Kopfdrüsen der geschwantzen Amphibien und die Glandula intermaxillaris der Anuren. Zeits. f. wissensch. Zool., Bd. XXVII.

Whipple, I. L.
'06 The Ypsiloid Apparatus of Urodeles. Biol. Bull., Vol. X., No. 6.

Wilder, H. H.
'91 Die Nasengegend von Menopoma alleghenienses und Amphiuma tridactylum. Zool. Jahrb., Bd. V.
'94 Lungenlose Salamandriden. Anat. Anz., Bd. IX., No. 7.